Research Article

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Nicotinoid and pyrethroid insecticide resistance in houseflies (Diptera: Muscidae) collected from Florida dairies

Phillip E Kaufman,[a*] Sonia C Nunez,[a] Rajinder S Mann,[a] Christopher J Geden[b] and Michael E Scharf[a]

Abstract

BACKGROUND: The housefly, *Musca domestica* L., continues to be a major pest of confined livestock operations. Houseflies have developed resistance to most chemical classes, and new chemistries for use in animal agriculture are increasingly slow to emerge. Five adult housefly strains from four Florida dairy farms were evaluated for resistance to four insecticides (beta-cyfluthrin, permethrin, imidacloprid and nithiazine).

RESULTS: Significant levels of tolerance were found in most field strains to all insecticides, and in some cases substantial resistance was apparent (as deduced from comparison with prior published results). At the LC_{90} level, greater than 20-fold resistance was found in two of the fly strains for permethrin and one fly strain for imidacloprid. Beta-cyfluthrin LC_{90} resistance ratios exceeded tenfold resistance in three fly strains. The relatively underutilized insecticide nithiazine had the lowest resistance ratios; however, fourfold LC_{90} resistance was observed in one southern Florida fly strain. Farm insecticide use and its impact on resistance selection in Florida housefly populations are discussed.

CONCLUSION: Housefly resistance to pyrethroids is widespread in Florida. Imidacloprid resistance is emerging, and tolerance was observed to both imidacloprid and nithiazine. If these insecticides are to retain efficacy, producer use must be restrained.

Keywords: Insecta; resistance; imidacloprid; nithiazine; fly bait; pyrethroid; *Musca domestica*

1 INTRODUCTION

The housefly, *Musca domestica* L., continues to be a major pest in livestock systems, particularly dairies, owing to its role in pathogen transmission, irritation to humans and animals and dispersal to off-farm areas. Houseflies have been shown to be resistant to most insecticides used against them, including permethrin and cyfluthrin.[1–3] Two insecticides have recently been formulated as baits for housefly control, imidacloprid and nithiazine. Studies have shown both of these active ingredients to be effective against field populations of houseflies, but early signs of resistance development with imidacloprid have been observed.[4,5]

Pesticide use on Florida dairies continues to rely on premise applications targeting adult houseflies; however, considerable applications are made with permethrin against horn flies, especially to the milking herd, as animals are often housed on pastures or open lots. The emergence and rapid adoption of the housefly baits QuickBayt™ (Bayer, Shawnee Mission, KS) and QuikStrike Fly Abatement Strip™ (Wellmark International, Schaumburg, IL) in the past 10 years provided dairy producers with effective options to combat adult houseflies. The active ingredients in these two products, imidacloprid and nithiazine respectively, provide a quick knockdown, which is visually appealing to a producer. However, within a few years, reports of reduced efficacy were received from producers and extension service personnel.

Previous insecticide resistance research has included Florida housefly strains, but the examination of these strains has been limited in scope.[4,6] Past research on New York dairies documented that resistance patterns were similar across the state.[7] In Florida dairy operations, the open architecture and management systems lend themselves to even more housefly dispersal opportunities, as most animals are housed outdoors.

Here, the results of the first statewide survey of housefly insecticide resistance in Florida are reported. This research was conducted with field-collected populations of houseflies by measuring the susceptibility levels to commonly used insecticides. The aim was to survey the levels of resistance in Florida dairies to allow for the development of a proactive insecticide resistance prevention program for the new active ingredients imidacloprid and nithiazine, and to provide baselines for future monitoring efforts.

* Correspondence to: Phillip E Kaufman, Entomology and Nematology Department, PO Box 110620, University of Florida, Gainesville, FL 32611, USA. E-mail: pkaufman@ufl.edu

a Entomology and Nematology Department, University of Florida, Gainesville, FL, USA

b USDA-ARS, Center for Medical, Agricultural and Veterinary Entomology, Gainesville, FL, USA

2 MATERIALS AND METHODS

2.1 Insects, farms and chemicals

Adult houseflies were collected by sweep net from within dairy barns and around calf areas on four Florida dairies in Alachua, Gilchrist, Lafayette and Okeechobee counties. Laboratory colonies of each strain were established from field isolates determined to be free of pathogens and ectoparasites. Houseflies were reared as previously described.[3] A laboratory strain, Florida susceptible (FS), of insecticide-susceptible houseflies served as the standard laboratory strain with which all field strains were compared. This strain was colonized in the late 1960s and has remained unchallenged since its collection.

The Alachua county farm was the University of Florida's dairy calf unit. Two fly strains were evaluated from this farm, a merged strain (UF 05–06) from two collections (December 2005 and January 2006) and a second strain (UF 07) collected in late 2007. Insecticide use at this farm consisted of permethrin-containing pour-ons applied to calves monthly, permethrin pour-ons on the adjacent paddocked heifers every 2 weeks and nithiazine baits (QuikStrike Fly Abatement Strip) during the housefly season (April–December). Houseflies were collected on the Gilchrist county farm in October 2007. This farm had used coumaphos (non-lactating animals) and permethrin as a pour-on, diazinon ear tags (non-lactating) over an extended timeframe and imidacloprid-containing QuickBayt Fly Bait intermittently at the calf area since 2004. The Lafayette county fly colony was established from fly collections in October 2007. This farm had heavy use of permethrin and imidacloprid. Permethrin was applied in three ways – through ear tags to cattle, as a daily spray to cattle and as a twice-weekly structure application – during the fly season. QuickBayt Fly Bait is applied as needed during non-rain times outside all cattle-holding buildings. The Okeechobee county fly strain was established in March 2008. This farm had previously used organophosphate ear tags (non-lactating cattle) on pastured cattle, pyrethroids applied as a fog in facilities and QuickBayt Fly Bait as needed in the barn areas.

Three technical-grade insecticides were tested: beta-cyfluthrin (99.5%; Chem Service, West Chester, PA), permethrin (99.2%, *cis : trans* 32 : 68; Chem Service, West Chester, PA) and imidacloprid (99.5%; Chem Service, West Chester, PA). One formulated insecticide was examined: nithiazine (Quikstrike 1.0% Fly Abatement Strip).

Nithiazine was extracted from the bait strip formulation using the following procedure. The formulation, a yellow bait matrix containing the nithiazine, was scraped from the bait strip paper support structure. The resultant mixture was weighed and placed in a 50 mL screw-top glass vial to which 50 mL of 100% ethanol was added. The test tube was covered in foil, placed on a rotisserie shaker (Barnstead International, Dubuque, IA) and rolled for 24 h. After rotation, the supernatant was transferred to a clean amber container for storage and served as the stock solution. The concentration of the stock solution was calculated by using the initial matrix weight and nithiazine percentage of the formulation that had been removed from the bait strip and the recovered volume of ethanol–nithiazine solution. Nithiazine recovery was verified by thin-layer chromatography using Whatman LHPKDF plates (Maidstone, UK) and a solvent system of dichloromethane + methanol (90 + 10 by volume). The entire process and subsequent storage was conducted in darkness to prevent nithiazine breakdown. Ethanol extracts were stored in amber bottles and held at 9 °C.

2.2 Laboratory bioassays

Permethrin and beta-cyfluthrin toxicities were examined using a residual contact method, while imidacloprid and extracted nithiazine were examined using a feeding assay. For the residual contact method, 20 adult female houseflies (3–5 days old) were placed inside a 60 mL glass jar (internal surface area 67.8 cm^2) that had been treated with technical-grade insecticide serially diluted in acetone to deliver a concentration–mortality range between 0 and 95%.

Imidacloprid and nithiazine do not penetrate the insect integument well and are commercially formulated as baits; therefore, a feeding assay was used to evaluate these insecticides. Female flies (20) were placed in plastic 500 mL containers and were provided with three 3.5 g sugar cubes (Dixie Crystals, Imperial-Savannah LP, Sugar Land, TX) each containing a serial dilution of either imidacloprid or nithiazine or a solvent-only control. Imidacloprid was serially diluted in acetone, and nithiazine was serially diluted in ethanol. The serially diluted solution or solvent-only control (0.5 mL) was applied to each sugar cube, and the solvent was allowed to evaporate. Flies were introduced into containers 1 h after treatment.

Between nine and 11 concentrations were used to generate concentration–response curves. For all insecticides at all concentrations, a minimum of 240 houseflies were tested. Bioassays were conducted at 26 °C with a 12 : 12 h light : dark photoperiod. Flies remained in residual contact bioassay jars for 48 h before mortality was assessed. During this holding period, flies were provided with a 20% sugar solution on a cotton wick. Flies in feeding bioassays were exposed to imidacloprid-treated sugar cubes for 96 h or nithiazine-treated sugar cubes for 24 h; mortality was assessed immediately following exposure. In feeding bioassays, flies were provided with water on a cotton wick. In both bioassays, flies were considered dead if they were ataxic.

The insecticide-susceptible FS strain was used to generate a complete concentration–response line for all fly strains with each insecticide. Bioassay data from three replications were pooled and analyzed by standard probit analysis,[8] as adapted to personal computer use by Raymond[9] using Abbott's transformation[10] to correct for control mortality. Resistance ratios, based on the FS strain standard, were determined using LC_{50} and LC_{90} values,[11] and all hypotheses were tested by the likelihood ratio test.[12]

3 RESULTS AND DISCUSSION

The highest levels of insecticide resistance were found with permethrin (Table 1). All field-collected strains examined were significantly more tolerant or resistant to permethrin than the FS strain, depending on the strain–insecticide combination, with the greatest resistance ratios (RR) observed in flies from the UF 07 ($RR_{90} = 21.3$) and Lafayette county strains ($RR_{90} = 22.7$). As reported earlier, the Lafayette county fly strain had extensive exposure to permethrin.

Significant levels of beta-cyfluthrin resistance were found in all housefly strains examined, with three of the LC_{90} resistance ratios at 10.0 or greater (Table 2). Adult houseflies from UF 07 strain demonstrated the highest level of beta-cyfluthrin resistance (14.4), whereas resistant ratios with UF 05–06 strain flies collected at the same farm 2 years earlier were significantly different from the FS strain, but had considerably lower LC values than the UF 07 strain.

The two university dairy strains collected 22 months apart from the same facility (UF 05–06 and UF 07) demonstrated a dramatic

Table 1. Toxicity of permethrin on glass to housefly adults from dairies in Florida

Strain	*n*	LC_{50} (µg cm^{-2}) (95% CI)	LC_{90} (µg cm^{-2}) (95% CI)	RR_{50}[a,c]	RR_{90}[b,c]	Slope (SE)
Florida susceptible	2400	0.13 (0.12–0.14)	0.33 (0.30–0.37)	–	–	3.3 (0.2)
UF 05-06[d]	2160	0.23 (0.21–0.25)	1.17 (1.02–1.37)	1.7*	3.6*	1.8 (0.1)
UF 07[d]	2160	1.54 (1.41–1.68)	6.98 (6.14–8.06)	11.5*	21.3*	1.9 (0.1)
Gilchrist	2400	0.82 (0.75–0.89)	3.53 (3.12–4.06)	6.1*	10.8*	2.0 (0.1)
Lafayette	2160	1.89 (1.73–2.06)	7.43 (6.58–8.53)	14.1*	22.7*	2.2 (0.1)
Okeechobee	1920	0.72 (0.64–0.80)	3.25 (2.78–3.90)	5.4*	9.9*	1.9 (0.1)

[a] Resistance ratio at LC_{50} (i.e. LC_{50} resistant strain/LC_{50} Florida susceptible).
[b] Resistance ratio at LC_{90} (i.e. LC_{90} resistant strain/LC_{90} Florida susceptible).
[c*] Significantly different from 1.0, based on non-overlap of 95% CI.
[d] University of Florida dairy, Alachua county, FL.

Table 2. Toxicity of beta-cyfluthrin on glass to housefly adults from dairies in Florida

Strain	*n*	LC_{50} (ng cm^{-2}) (95% CI)	LC_{90} (ng cm^{-2}) (95% CI)	RR_{50}[a,c]	RR_{90}[b,c]	Slope (SE)
Florida susceptible	1700	3.86 (3.48–4.29)	12.54 (10.66–15.31)	–	–	2.5 (0.2)
UF 05-06[d]	1920	8.17 (7.34–9.08)	34.60 (29.55–41.60)	2.1*	2.8*	2.0 (0.1)
UF 07[d]	1920	35.12 (32.02–38.52)	180.13 (155.62–212.49)	9.1*	14.4*	1.8 (0.1)
Gilchrist	2400	14.22 (12.84–15.72)	66.76 (57.72–78.69)	3.7*	5.3*	1.9 (0.1)
Lafayette	1920	29.92 (27.47–32.57)	124.89 (110.05–143.93)	7.7*	10.0*	2.1 (0.1)
Okeechobee	1920	27.57 (25.08–30.28)	148.80 (128.55–175.45)	7.1*	11.9*	1.8 (0.1)

[a] Resistance ratio at LC_{50} (i.e. LC_{50} resistant strain/LC_{50} Florida susceptible).
[b] Resistance ratio at LC_{90} (i.e. LC_{90} resistant strain/LC_{90} Florida susceptible).
[c*] Significantly different from 1.0, based on non-overlap of 95% CI.
[d] University of Florida dairy, Alachua county, FL.

increase in permethrin and beta-cyfluthrin tolerance, with LC_{90} values rising from 1.17 to 6.98 for permethrin and from 34.60 to 180 for beta-cyfluthrin. Such a dramatic rise in tolerance suggests prior selection with pyrethroids or perhaps a collection of flies in 2007 shortly after pyrethroid exposure.

All Florida housefly strains examined were significantly resistant to imidacloprid; however, some resistance ratios remain relatively low (Table 3). The Lafayette county dairy houseflies carried the largest resistance level, with an RR_{90} of 23.7. Resistance ratios from the remaining dairies were considerably lower. All were significant, however, suggesting that there is a strong potential for loss of imidacloprid efficacy with continued selection.

Three of the five housefly stains demonstrated significant differences in nithiazine tolerance/resistance as compared with the FS strain at the LC_{50}, and four of the five strains demonstrated significant tolerance/resistance at the LC_{90} (Table 4). Also, interestingly, the Gilchrest county dairy flies were significantly more susceptible at the LC_{50} than the susceptible laboratory strain. It is not surprising that nithiazine tolerance levels are much lower than the tolerance levels of the other three insecticides examined because of the method of application for this insecticide and its relatively lower use by producers. Nithiazine is sold as the QuikStrike Fly Abatement Strip, which is a prefabricated device wherein the chemical is applied to a strip that is placed within a plastic housing. Because the chemical breaks down readily in sunlight, it must be used indoors or in sunlight-protected outdoor areas. In contrast, the imidacloprid product (QuickBayt) is a granular bait that is scattered in areas that flies frequent and has been utilized by producers much more frequently. To the authors' knowledge, the University of Florida dairy was the only facility regularly to use the QuikStrike Fly Abatement Strip. However, there were no appreciable susceptibility differences between farms that reported no use of nithiazine. It appears that nithiazine is still an efficacious insecticide against houseflies in Florida.

It is evident from the present results that houseflies in Florida have acquired tolerance, and in some cases resistance, to insecticides used against them. However, field efficacy may still be present. Here, tolerance was categorized as a non-significant increase in the resistance ratio, while resistant populations were categorized as having significant resistance ratios. Previously, Kaufman *et al.*[7] identified excellent linkage between laboratory studies and field efficacy; in particular, significant resistance ratios were found to be associated with field control failures. While these studies are labor intensive, they appear to provide an excellent measure of resistance potential.

Two previous studies measured the susceptibility of imidacloprid in the laboratory[4] and nithiazine in the field (QuikStrike strip) on two Florida dairies that were approximately 15 km from the Gilchrist county dairy used in the present study.[13] The houseflies collected in 2004 from one of these dairies were found to be susceptible to imidacloprid, with an LC_{50} value of 94 µg mL^{-1} and an RR of 3.1.[4] It was not possible to collect flies at either of these dairies, as neither were in operation in 2007. The closest dairy in the present study to those mentioned above was the Gilchrist county dairy, with an LC_{50} value of 60 µg g^{-1} sugar and an RR of 3.3. However, in the present assay, flies were allowed to feed for 96 h, as opposed to 72 h in the Kaufman[4] study. The extended feeding in the present study would be expected to lower LC values and limit the observed magnitude of tolerance/resistance. Nevertheless, the resistance ratios were quite similar, suggesting

Table 3. Toxicity of imidacloprid fed in a sugar bait to housefly adults from dairies in Florida

Strain	*n*	LC_{50} ($\mu g\ g^{-2}$) (95% CI)	LC_{90} ($\mu g\ g^{-2}$) (95% CI)	RR_{50} [a,c]	RR_{90} [b,c]	Slope (SE)
Florida susceptible	1920	18 (15.5–21)	65 (52–89)	–	–	2.3 (0.2)
UF 05-06[d]	1760	38 (34–43)	143 (122–173)	2.1*	2.2*	2.2 (0.1)
UF 07[d]	2160	77 (68–87)	446 (369–555)	4.3*	6.8*	1.7 (0.1)
Gilchrist	1920	60 (50–70)	346 (273–462)	3.3*	5.3*	1.7 (0.1)
Lafayette	2160	231 (207–258)	1550 (1276–1941)	12.8*	23.7*	1.6 (0.1)
Okeechobee	1920	43 (37–50)	202 (159–274)	2.4*	3.1*	1.9 (0.1)

[a] Resistance ratio at LC_{50} (i.e. LC_{50} resistant strain/LC_{50} Florida susceptible).
[b] Resistance ratio at LC_{90} (i.e. LC_{90} resistant strain/LC_{90} Florida susceptible).
[c]* Significantly different from 1.0, based on non-overlap of 95% CI.
[d] University of Florida dairy, Alachua county, FL.

Table 4. Toxicity of nithiazine fed in a sugar bait to housefly adults from dairies in Florida

Strain	*n*	LC_{50} ($\mu g\ g^{-2}$) (95% CI)	LC_{90} ($\mu g\ g^{-2}$) (95% CI)	RR_{50} [a,c]	RR_{90} [b,c]	Slope (SE)
Florida susceptible	1920	0.46 (0.44–0.49)	1.04 (0.93–1.19)	–	–	3.6 (0.2)
UF 05-06[d]	1920	0.49 (0.44–0.55)	1.87 (1.58–2.29)	1.1	1.8*	2.2 (0.1)
UF 07[d]	1920	0.66 (0.59–0.73)	2.53 (2.15–3.07)	1.4*	2.4*	2.2 (0.1)
Gilchrist	1920	0.27 (0.25–0.31)	0.85 (0.73–1.00)	0.6*	0.8	2.6 (0.1)
Lafayette	1920	0.98 (0.89–1.08)	4.04 (3.44–4.85)	2.1*	3.9*	2.1 (0.1)
Okeechobee	1920	0.99 (0.87–1.13)	4.31 (3.51–5.52)	2.1*	4.1*	2.0 (0.1)

[a] Resistance ratio at LC_{50} (i.e. LC_{50} resistant strain/LC_{50} Florida susceptible).
[b] Resistance ratio at LC_{90} (i.e. LC_{90} resistant strain/LC_{90} Florida susceptible).
[c]* Significantly different from 1.0, based on non-overlap of 95% CI.
[d] University of Florida dairy, Alachua county, FL.

that imidacloprid resistance selection of houseflies in this part of Florida had not changed appreciably between 2004 and 2007.

The pyrethroid resistance levels observed here are considerable and reflect a higher level than had previously been reported. Marcon *et al.*[14] reported sevenfold permethrin resistance in houseflies from Nebraska, and Kaufman *et al.*[7] documented >90% survival of New York dairy-farm-collected, cyfluthrin-exposed houseflies to the $3\times$ LC_{99} level ($LC_{99} = 8.3$ ng cm^{-2}), suggesting an RR approaching 3.0. The present results document that Florida housefly populations have exceeded the New York levels, and in some cases the Nebraska levels, and are becoming increasingly cross-resistant to the newer pyrethroids.

Housefly exposure to pyrethroids may be different under Florida animal husbandry conditions. In other areas of the United States it is common for producers to treat walls and other structures with a pyrethroid residual spray. In Florida, although pesticide applications to structures are much less common owing to the open architecture of buildings, the use of pour-on formulations is frequent. Moreover, because many cattle are pastured or placed in paddocks during the day, houseflies are regularly observed resting on animals or feeding on their secretions while out-of-doors. This is increasingly common under dry conditions where the animals often serve as a source for water. To this extent, the exposure of houseflies to pyrethroids applied to animals as pour-ons may be facilitated by these regional conditions.

Imidacloprid and, to a lesser extent, nithiazine appear to be losing their effectiveness, and the continued widespread use of imidacloprid warrants concern. That resistance is greater in Florida may be partially explained by the longer housefly season and relative dispersal of confined dairy operations in the state. Heavy usage of insecticides by a producer would likely drive a population towards greater resistance, especially in the absence of susceptible, resistance-diluting populations on nearby farms. Therefore, the authors suggest that the situation in Florida is similar to the housefly resistance patterns observed in New York poultry operations.[3] In these studies, insecticide application practices in enclosed poultry operations were more directly related to pesticide resistance expression than in the open, and to more clustered dairies in that state where resistance expression had little to do with producer applications (owing to fly movement between the open farms).[3,7]

Although resistance to insecticide baits has been reported in many insect species, few reports of resistance exist for houseflies. Insecticide bait applications have been considered low-impact drivers of resistance in houseflies owing to the great mobility exhibited by houseflies and the wide range of acceptable food substrates. Recent work in California[5] and New York[3,7] has documented that houseflies have developed resistance to methomyl. The present results with imidacloprid and nithiazine suggest that baits containing these toxicants are vulnerable to resistance development when used heavily.

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